Periphyton Chlorophyll-*a* Monitoring in Support of Stream Nutrient Criteria Development

FY 02 104(b)(3) CA#X976045-01-0

1 Dec 2004

Water Quality Programs Division
Oklahoma Water Resources Board

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INTRODUCTION

Each state has been directed by EPA to promulgate nutrient criteria. Excluding Scenic Rivers, Oklahoma plans to establish nutrient criteria based upon thresholds protective of Beneficial Uses rather than to adopt the percentile-based criteria suggested for each nutrient ecoregion. Periphyton may serve as an appropriate end point criteria for controlling impairments caused by nutrients. Although algae levels may not be easily implemented through permits, they definitely have a direct bearing on beneficial use support and therefore should be monitored. Prior to this project, Oklahoma monitoring programs have not included *in situ* measurements of stream algae or phytoplankton as part of any widespread monitoring program. Consequently, data available to establish nutrient criteria based upon Beneficial Use protection are limited. To establish nutrient criteria to control nuisance algae levels in Oklahoma streams, some determination of what a nuisance level is must be made. Similarly, nutrient criteria to protect the Fish and Wildlife Beneficial Use should be related to algae in the stream as well as impairments of the aquatic community. The objective of this project was to initiate periphyton collection as part of routine monitoring of Oklahoma streams. This report conveys the data collected with this grant. Future analysis of the data collected with this project and routine periphyton monitoring initiated as result of this project will serve in nutrient criteria development.

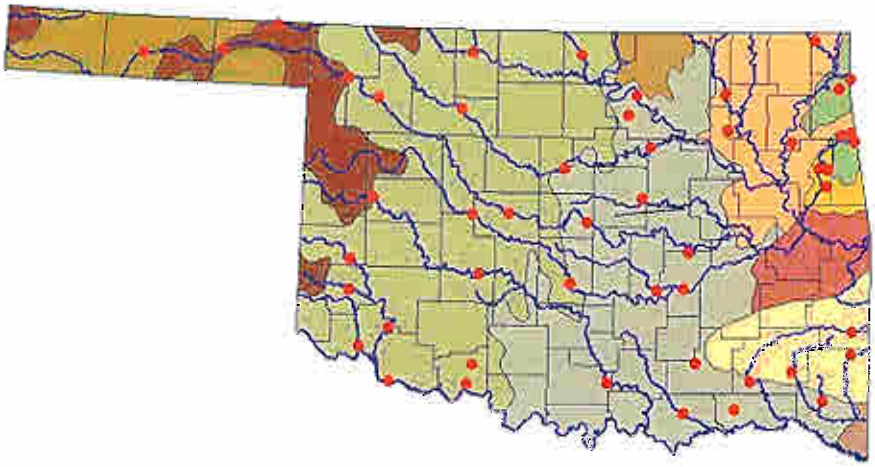
MATERIALS AND METHODS

Phytoplankton and periphyton samples were collected following protocols modified from those established by the EPA Environmental Monitoring and Assessment Program (EMAP) and the USGS National Water Quality Assessment Program (NAWQA) programs. The periphyton collection protocol follows Hill (2001) and calls for a composite of 11 samples taken from a stream reach of 20 times the stream width. The minimum reach assessed was 200 m with samples taken at 20 m increments. The maximum reach assessed was 800 m with samples collected at 80 m increments. The sestonic chlorophyll is collected as a composite, depth-integrated sample. The OWRB standard operating procedure (SOP) for stream chlorophyll collection is attached in appendix 1. The periphyton SOP and monitoring strategy for this project were added to the OWRB Beneficial Use Monitoring Program Quality Assurance Project Plan (QAPP). The QAPP addendum was approved by EPA Region Six in October 2002. The specific water-bodies initially considered for sampling are listed in Table 1 and are depicted in Figure 1.

Table 1. *Waters proposed for monitoring.*

No.	Waterbody ID	Name
1	OK621200010200	ARKANSAS RIVER, SH 18, RALSTON
2	OK121700050010	BARREN FORK, SH 51, ELDON
3	OK720510000190	BEAVER RIVER, OFF US 64, GUYMON
4	OK720500020010	BEAVER RIVER, US 183, FORT SUPPLY
5	OK720500020450	BEAVER RIVER, US 83, TURPIN
6	OK121300010010	BIRD CREEK, SH 266, PORT OF CATOOSA
7	OK621200030010	BLACK BEAR CREEK, SH 18, PAWNEE
8	OK410600010010	BLUE RIVER, US 70, DURANT
9	OK220600010119	CANADIAN RIVER, US 270, CALVIN
10	OK520610020150	CANADIAN RIVER, US 281, NEAR BRIDGEPORT
11	OK520610010010	CANADIAN RIVER, US 77, PURCELL
12	OK121700040010	CANEY CREEK,, OFF SH 100, BARBER
13	OK121400010010	CANEY RIVER, OFF US 75, RAMONA
14	OK621100000010	CHICKASKIA RIVER, US 177, BLACKWELL
15	OK620900010170	CIMARRON RIVER, SH 99, OILTON
16	OK620910010010	CIMARRON RIVER, US 77, GUTHRIE
17	OK620930000010	CIMMARON RIVER, OFF US 64, MOCANE
18	OK620920010010	CIMMARON RIVER, US 412, ORIENTA
19	OK520700040010	DEEP FORK, US 377, STROUD
20	OK311300010020	EAST CACHE CREEK, SH 53, WALTERS
21	OK121600030440	ELK RIVER, SH 43, TIFF CITY (MO)
22	OK311800000010	ELM FORK RIVER, SH 9, MANGUM
23	OK121700060010	FLINT CREEK, US 412, KANSAS
24	OK410210080010	GLOVER RIVER, SH 3, GLOVER
25	OK121600030440	HONEY CREEK, OFF SH 25, GROVE
26	OK121700030350	ILLINOIS RIVER, US 59, WATTS
27	OK121700030010	ILLINOIS RIVER, US 62, TAHLEQUAH
28	OK410310020010	KIAMICHI RIVER, SH 63, BIG CEDAR
29	OK410300030010	KIAMICHI RIVER, US 271, ANTLERS
30	OK410210020140	LITTLE RIVER, OFF SH 3, CLOUDY
31	OK520800010010	LITTLE RIVER, SH 56, SASAKWA
32	OK410210060010	MOUNTAIN FORK, SH 4, SMITHVILLE
33	OK410400010070	MUDDY BOGGY CREEK, US 70, UNGER
34	OK410400050270	MUDDY BOGGY RIVER, US 69, ATOKA
35	OK121600040220	NEOSHO RIVER, OFF US 66, COMMERCE
36	OK520510000110	NORTH CANADIAN RIVER, OFF US 62, HARRAH
37	OK720500010140	NORTH CANADIAN RIVER, US 412, WOODWARD
38	OK520510000010	NORTH CANADIAN RIVER, US 75, WETUMKA
39	OK520530000010	NORTH CANADIAN RIVER, US 81, EL RENO
40	OK311500010020	NORTH FORK OF RED RIVER, US 62, HEADRICK
41	OK311510010010	NORTH FORK OF THE RED RIVER, SH 34, CARTER
42	OK311310010010	RED RIVER, US 183, DAVIDSON
43	OK121700060080	SAGER CREEK, OFF US 412, WEST SILOAM SPRINGS
44	OK621010010160	SALT FORK OF ARKANSAS, SH 58, INGERSOL
45	OK311600020010	SALT FORK OF THE RED RIVER, OFF US 283, ELMER
46	OK121600010290	SPRING CREEK, OFF US 412, MURPHY
47	OK310840010010	WASHITA RIVER, SH 33, HAMMON
48	OK310800020010	WASHITA RIVER, US 177, DURWOOD
49	OK310830010010	WASHITA RIVER, US 281, ANADARKO
50	OK311310020010	WEST CACHE CREEK, SH 5B, TAYLOR

Figure 1. Waters proposed for monitoring.



RESULTS

Chlorophyll-a

The periphyton and sestonic chlorophyll-*a* data collected during the summer of 2003 as part of this project are tabulated in table 2. When streams were not wadable, no periphyton collections were attempted. It was not possible to calculate benthic chlorophyll for samples collected in month of June due to missing sample volume data. Benthic Chlorophyll samples were also not collected from non wadable streams or during high flow.

Table 2 Benthic and sestonic chlorophyll collected at selected sites summer 2003

Waterbody Segment ID	**Station Description**	**Sample Date**	Benthic chlorophyll a mg/m^2	Sestonic chlorophyll a ug/l
121500030010-001AT	VERDIGRIS RIVER, SH 20, KEETONVILLE	7/22/03		7.1
121500030010-001AT	VERDIGRIS RIVER, SH 20, KEETONVILLE	8/25/03		6.5
121510020010-001AT	VERDIGRIS RIVER, SH 10, LENEPAH	7/22/03		19.7
121510020010-001AT	VERDIGRIS RIVER, SH 10, LENEPAH	8/25/03		3.8

Waterbody Segment ID	**Station Description**	**Sample Date**	Benthic chlorophyll a mg/m^2	Sestonic chlorophyll a ug/l
121600010290-001AT	SPRING CREEK, OFF US 412, MURPHY	6/17/03		0.6
121600010290-001AT	SPRING CREEK, OFF US 412, MURPHY	7/22/03	5.3	0.7
121600010290-001AT	SPRING CREEK, OFF US 412, MURPHY	8/25/03	5.5	0.2
121600030290-001AT	HONEY CREEK, OFF SH 25, GROVE	6/16/03		1.2
121600030290-001AT	HONEY CREEK, OFF SH 25, GROVE	7/21/03	24.1	
121600030290-001AT	HONEY CREEK, OFF SH 25, GROVE	7/22/03		0.6
121600030290-001AT	HONEY CREEK, OFF SH 25, GROVE	8/26/03	43.7	1.4
121600030440-001AT	ELK RIVER, SH 43, TIFF CITY (MO)	6/16/03		2.5
121600030440-001AT	ELK RIVER, SH 43, TIFF CITY (MO)	7/21/03	92.1	4.2
121600030440-001AT	ELK RIVER, SH 43, TIFF CITY (MO)	8/26/03	39.5	1.3
121600040220-001AT	NEOSHO RIVER, OFF US 66, COMMERCE	7/21/03		2.7
121600040220-001AT	NEOSHO RIVER, OFF US 66, COMMERCE	8/26/03		3.6
121600060060-001AT	BIG CABIN CREEK, OFF US 69, BIG CABIN	7/22/03		61.3
121600060060-001AT	BIG CABIN CREEK, OFF US 69, BIG CABIN	8/25/03		25.1
121600070010-001AT	SPRING RIVER, OFF SH 137, QUAPAW	7/21/03		13.5
121600070010-001AT	SPRING RIVER, OFF SH 137, QUAPAW	8/26/03		1.6
121700030010-001AT	ILLINOIS RIVER, US 62, TAHLEQUAH	6/18/03		3.7
121700030010-001AT	ILLINOIS RIVER, US 62, TAHLEQUAH	7/23/03	19.4	1.9
121700030010-001AT	ILLINOIS RIVER, US 62, TAHLEQUAH	8/26/03	44.9	2.6
121700030350-001AT	ILLINOIS RIVER, US 59, WATTS	6/17/03		3.5
121700030350-001AT	ILLINOIS RIVER, US 59, WATTS	7/22/03	19.2	3.9
121700030350-001AT	ILLINOIS RIVER, US 59, WATTS	8/25/03	58.4	0.1
121700040010-001AT	CANEY CREEK, OFF SH 100, BARBER	6/18/03		1.2
121700040010-001AT	CANEY CREEK, OFF SH 100, BARBER	7/23/03	10.6	3.1
121700040010-001AT	CANEY CREEK, OFF SH 100, BARBER	8/26/03	17.8	0.7
121700050010-001AT	BARREN FORK, SH 51, ELDON	6/17/03		2.1
121700050010-001AT	BARREN FORK, SH 51, ELDON	7/23/03	18.2	1.1
121700050010-001AT	BARREN FORK, SH 51, ELDON	8/26/03	28.4	0.5
121700060010-001AT	FLINT CREEK, US 412, FLINT	7/22/03		0.9
121700060010-001AT	FLINT CREEK, US 412, FLINT	8/25/03	15.3	1.8
121700060080-001AT	SAGER CREEK, OFF US 412, WEST SILOAM SPRINGS	6/17/03		0.7

Waterbody Segment ID	**Station Description**	**Sample Date**	Benthic chlorophyll a mg/m^2	Sestonic chlorophyll a ug/l
121700060080-001AT	SAGER CREEK, OFF US 412, WEST SILOAM SPRINGS	7/22/03	2.1	0.6
121700060080-001AT	SAGER CREEK, OFF US 412, WEST SILOAM SPRINGS	8/25/03	29.0	0.4
220600010119-001AT	CANADIAN RIVER, US 270, CALVIN	8/5/03		21.4
220600010119-001AT	CANADIAN RIVER, US 270, CALVIN	9/10/03		25.5
310830010010-001AT	WASHITA RIVER, US 281, ANADARKO	8/20/03	4.5	14.4
310840010010-001AT	WASHITA RIVER, SH 33, HAMMON	8/20/03	15.0	10.8
311300010020-001AT	EAST CACHE CREEK, SH 53, WALTERS	6/4/03		7.3
311300010020-001AT	EAST CACHE CREEK, SH 53, WALTERS	8/19/03		1.1
311310020010-001AT	WEST CACHE CREEK, SH 5B, TAYLOR	6/4/03		8.3
311310020010-001AT	WEST CACHE CREEK, SH 5B, TAYLOR	8/19/03		1.6
311500010020-001AT	NORTH FORK OF RED RIVER, US 62, HEADRICK	6/3/03		25.5
311500010020-001AT	NORTH FORK OF RED RIVER, US 62, HEADRICK	8/19/03	7.9	12.3
311510010010-001AT	NORTH FORK OF THE RED RIVER, SH 34, CARTER	6/2/03		25.4
311800000010-001AT	ELM FORK RIVER, SH 9, MANGUM	6/3/03		6.3
311800000010-001AT	ELM FORK RIVER, SH 9, MANGUM	8/20/03	19.1	7.2
410210020140-001AT	LITTLE RIVER, OFF SH 3, CLOUDY	5/20/03		3
410210020140-001AT	LITTLE RIVER, OFF SH 3, CLOUDY	7/30/03	12.8	0.8
410210020140-001AT	LITTLE RIVER, OFF SH 3, CLOUDY	9/3/03		4.4
410210060010-001AT	MOUNTAIN FORK RIVER, SH 4, SMITHVILLE	5/19/03		2.1
410210060010-001AT	MOUNTAIN FORK RIVER, SH 4, SMITHVILLE	7/29/03	8.3	3.6
410210060010-001AT	MOUNTAIN FORK RIVER, SH 4, SMITHVILLE	9/2/03		3.6
410210080010-001AT	GLOVER RIVER, SH3, GLOVER	5/20/03		2.2
410210080010-001AT	GLOVER RIVER, SH3, GLOVER	7/29/03	27.6	2.1
410210080010-001AT	GLOVER RIVER, SH3, GLOVER	9/3/03		2.2
410310020010-001AT	KIAMICHI RIVER, SH 63, BIG CEDAR	5/19/03		0.8
410310020010-001AT	KIAMICHI RIVER, SH 63, BIG CEDAR	7/29/03	7.6	3.1
410310020010-001AT	KIAMICHI RIVER, SH 63, BIG CEDAR	9/2/03		1.7
520510000010-001AT	NORTH CANADIAN RIVER, US 75,	8/6/03		22.3

Waterbody Segment ID	**Station Description**	**Sample Date**	Benthic chlorophyll a mg/m^2	Sestonic chlorophyll a ug/l
	WETUMKA			
520510000110-001AT	NORTH CANADIAN RIVER, OFF US 62, HARRAH	8/6/03		13.3
520510000110-005AT	NORTH CANADIAN RIVER, SH 3E, SHAWNEE	8/6/03		84.1
520530000010-001AT	NORTH CANADIAN RIVER, US 81, EL RENO	6/30/03	3.4	32.0
520530000010-001AT	NORTH CANADIAN RIVER, US 81, EL RENO	8/6/03	0.1	4.5
520600010010-001AT	CANADIAN RIVER, US 377, KONAWA	8/5/03	13.2	19.9
520600010010-001AT	CANADIAN RIVER, US 377, KONAWA	9/10/03	11.1	35.4
520610010010-001AT	CANADIAN RIVER, US 77, PURCELL	8/5/03	25.8	47.3
520610010010-001AT	CANADIAN RIVER, US 77, PURCELL	9/10/03	19.8	36.0
520610020150-001AT	CANADIAN RIVER, US 66, BRIDGEPORT	6/30/03	1.5	18.1
520610020150-001AT	CANADIAN RIVER, US 66, BRIDGEPORT	8/5/03		5.5
520610020150-001AT	CANADIAN RIVER, US 66, BRIDGEPORT	8/6/03	14.9	
520700040010-001AT	DEEP FORK RIVER, US 377, STROUD	6/16/03		15.6
520700040010-001AT	DEEP FORK RIVER, US 377, STROUD	7/21/03	9.2	11.7
520700040010-001AT	DEEP FORK RIVER, US 377, STROUD	8/27/03	22.4	11.6
520800010010-001AT	LITTLE RIVER, SH 56, SASAKWA	8/5/03	4.4	16.5
520800010010-001AT	LITTLE RIVER, SH 56, SASAKWA	9/10/03	4.9	
620910010010-001AT	CIMARRON RIVER, US 77, GUTHRIE	6/10/03		24.2
620910010010-001AT	CIMARRON RIVER, US 77, GUTHRIE	6/11/03		25.9
620910010010-001AT	CIMARRON RIVER, US 77, GUTHRIE	7/7/03	3.0	7.6
620910010010-001AT	CIMARRON RIVER, US 77, GUTHRIE	8/11/03	44.9	15.6
620920010010-001AT	CIMMARON RIVER, US 412, ORIENTA	7/8/03	15.7	10.9
620920010010-001AT	CIMMARON RIVER, US 412, ORIENTA	8/11/03	16.1	11.0
620930000010-001AT	CIMMARON RIVER, OFF US 64, MOCANE	5/28/03		10.1
620930000010-001AT	CIMMARON RIVER, OFF US 64, MOCANE	5/28/03		9.8
620930000010-001AT	CIMMARON RIVER, OFF US 64, MOCANE	5/28/03		10.8
620930000010-001AT	CIMMARON RIVER, OFF US 64, MOCANE	7/1/03	6.7	6.6
620930000010-001AT	CIMMARON RIVER, OFF US 64, MOCANE	8/5/03	15.5	26.6

Waterbody Segment ID	**Station Description**	**Sample Date**	Benthic chlorophyll a mg/m^2	Sestonic chlorophyll a ug/l
621010010160-001AT	SALT FORK OF THE ARKANSAS RIVER, SH 58, INGERSOL	6/10/03		11.2
621010010160-001AT	SALT FORK OF THE ARKANSAS RIVER, SH 58, INGERSOL	7/8/03	7.8	2.8
621010010160-001AT	SALT FORK OF THE ARKANSAS RIVER, SH 58, INGERSOL	8/12/03	31.6	2.6
621100000010-001AT	CHICKASKIA RIVER, US 177, BLACKWELL	6/11/03		1.7
621100000010-001AT	CHICKASKIA RIVER, US 177, BLACKWELL	7/9/03		20.9
621100000010-001AT	CHICKASKIA RIVER, US 177, BLACKWELL	8/13/03		17.3
720500010140-001AT	NORTH CANADIAN RIVER, US 412, WOODWARD	5/27/03		12.3
720500010140-001AT	NORTH CANADIAN RIVER, US 412, WOODWARD	7/2/03	19.0	4.5
720500010140-001AT	NORTH CANADIAN RIVER, US 412, WOODWARD	8/6/03	86.2	5.8
720500020010-002AT	BEAVER RIVER, US 183, FORT SUPPLY	5/27/03		4.8
720500020010-002AT	BEAVER RIVER, US 183, FORT SUPPLY	7/2/03	31.4	2.6
720500020010-002AT	BEAVER RIVER, US 183, FORT SUPPLY	8/6/03	34.9	0.6
720510000190-001AT	BEAVER RIVER, OFF US 64, GUYMON	5/28/03		1.8
720510000190-001AT	BEAVER RIVER, OFF US 64, GUYMON	7/1/03	16.4	2.4
720510000190-001AT	BEAVER RIVER, OFF US 64, GUYMON	8/5/03	4.3	1.8

Photodocumentation:
Photodocumentation was planned for each sites and each sampling event. The availability of cameras and staff time limited the number of sites and sampling event photographed. An album of selected photos from the 2003 field season is included as appendix 2 of this report.

Discussion

Because of the limited data for each site and limited time frame of the data, only limited data analysis is presented at this time. With exception of a sample collected from the Elk River and a sample form the North Canadian River no samples approach the level of nuisance conditions established by Welch et al (1988). Average *chl-a* values for 60% of the sites with multiple samples were below the suggested threshold oligotrophic / mesotrophic boundary of 20 mg/m^2 suggested by Dodds et al.(1988). The maximum benthic chlorophyll threshold oligotrophic / mesotrophic boundary of 60 mg/m^2 suggested by Dodds et al (1988) was exceeded by only 4% of the samples.

This project helped institute periphyton monitoring as part of the OWRB routine monitoring program. The level of effort required to collect and process the samples limits the number of sites sampled. However, the importance of monitoring stream periphyton and sestonic algae has been realized. The 2004 summer season resulted in 15 sites sampled 2 – 3 times with only state funding. The 2004 effort focused principally of wadable streams and the state's scenic

rivers. A similar level of effort is anticipated for the 2005 season. Fall winter and spring sampling is under consideration.

The extent of periphyton monitoring may evolve as more practical and less labor intensive methods are identified. More rapid methods of assessing only index habitats and more efficient sampling devices are under evaluation.

Photos will be a critical tool for gaining public support for nutrient criteria. Photo documentation of periphyton associated with specific levels of periphyton biomass will be necessary to demonstrate the purpose and intended effect of nutrient criteria. Equipping assessment crews with adequate cameras and developing an "eye" for getting representative photos proved to be a challenge. Additonal techniques and equipment will be evaluated with future monitoring.

References

Hill, B. H., "Periphyton", EMAP Western Pilot Study Field Operations Manual for Wadeable Streams, 156-158 (2001).

Dodds, W.K, Jones J. R. and Welch, E. B; "Suggested classification of streams tropic state distributions of temperate stream types by chlorophyll, total nitrogen, and Phosphorus" (1988) *Water Research* 32, 1455-1462

Welch, E. B., J. M. Jacoby, R. R. Horner, and M. R. Seeley. 1988. Nuisance levels of periphytic algae in streams. Hydrobiologia 157:161–168.

APPENDIX 1

STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF BENTHIC AND SESTONIC CHLOROPHYLL-a SAMPLES IN STREAMS

1.0 Introduction

The purpose of this document is to provide a simplified, step-by-step outline of the field and laboratory procedures used by the Water Quality Programs Division of the Oklahoma Water Resources Board (OWRB) for the collection of benthic and sestonic chlorophyll-*a* in wadable rivers and streams. The basic sampling procedures that will be discussed in this document involve water quality sampling, methods and equipment. All documents needed for, including chain of custody forms and laboratory login sheets for both the OWRB and the Oklahoma Department of Environmental Quality (ODEQ), field data sheets, and checklists can be found at the end of this document.

2.0 Definitions/Terms

3.0 Safety

Upon reaching the sampling location, site safety determinations should be made before proceeding. These will be different for wadeable and bridge sites. Please refer to the OWRB safety manual for instructions on how to sample both kinds of sites. When regulating the flow of traffic is necessary, please refer to the portion of the safety manual outlining "Traffic Safety Protocols".

4.0 Quality of the Measurement

When sampling for all programs, Quality Assurance/Quality Control (QA/QC) samples will be routinely collected to assure that environmental samples meet the Data Quality Objectives (DQO's) that are outlined in the controlling Quality Assurance Project Plan (QAPP). QA/QC sampling is designed to control each step of the sampling process. Blanks are collected to ensure that field personnel are properly cleaning the plastics and glassware used in field sampling. Duplicate samples are collected to ensure that composite samples are properly processed. Replicate samples may be collected to ensure that the sampling methodology employed is collecting a representative sample. Spike or known samples may be submitted to test the efficacy of the analytical laboratory. The QA/QC protocols for sestonic chlorophyll-a can be found in the document "Standard Operating Procedure for the Collection of Water Quality Samples". The QA/QC samples for benthic chlorophyll-a are the same. However, since both collection and filtration equipment are both used more than once in the field, two code "33" samples should be taken—one for collection equipment and one for filtration equipment.

5.0 Personnel and Equipment

Principle investigators for the OWRB are required to have degrees and/or experience with biological or other applicable sciences. Principle investigators are defined as crew leaders, and this designation may be made upon the leader of a multi- or a one person crew. Training is required for all SOPs dealing with water quality and quantity collections and measurements as well as habitat assessments and biological collections. In-house training will be conducted for the use of all meters and digital titrators used for water quality or quantity measurements. Investigators must be familiar with OWRB SOP document and all training will follow the methods outlined in that document. Extra training will be provided when new SOPs are developed. Training of field crews will be done through dry run exercises in the laboratory to familiarize field crews with sample collection, sample preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods developed, qualified staff will train on sample collection, measurement, and field analysis methods through side-by-side field trips. These trips will familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will check field staff for adherence to SOPs.

In most instances, the collection of water quality samples requires only one field person. However, depending on the safety requirements of a particular station, additional crewmembers may be necessary to ensure a safe work zone. Equipment used to collect the chlorophyll-a sample are described in the document "Standard Operating Procedure for the Collection of Water Quality Samples".

5.1 Collection Equipment

For sestonic samples, the collection equipment is described in "Standard Operating Procedures for the Collection of Water Quality Samples". When collecting sestonic samples, an additional clean 1-L sample bottle labeled for chlorophyll-a should be included. The sestonic sample is merely collected from the splitter churn as an additional composite sample. To ensure cross-contamination has not occurred, a field blank (QA code 33) should be processed when sestonic chlorophyll-a samples are collected.

For benthic samples, the field collection unit should accompany the field crew. This unit includes a 1 or 2 gallon calibrated wide mouth jug, a large funnel, hard and soft substrate delimiters, coarse scrubbing brush, knife, spatula, rinse bottle, hip chain (extra string and stake), camera, and calculator. All parts should be cleaned thoroughly before leaving the office and while in the field. To ensure cleanliness, both laboratory (code 32) and field (code 33) blanks should be collected using all equipment coming into contact with the sample.

5.2 Filtration Equipment

A field filtration unit should accompany a field crew when benthic and sestonic chlorophyll-a collections are being made. The unit should be cleaned thoroughly after each use. This unit is composed of a filtration apparatus, glass fiber or membrane filters (0.45μm porosity, 47-mm diameter), rinse bottle, foil, marker, forceps, 250-mL plastic graduated cylinder, and zip-lock baggies. The filtration apparatus should include

a glass filter funnel and base, a plastic or glass vacuum beaker (1000 mL), vacuum tubing, and hand pump. All glass and plastic parts should be thoroughly cleaned before leaving for the field. To ensure cleanliness, a laboratory blank (QA code 32) should be filtered and processed. Vacuum tubing should be checked regularly for cracks, and the hand pump should be regularly checked to ensure that proper pressure can be regulated.

5.3 Extraction Equipment

For benthic samples, only chemical extraction is used. A clean and labeled 100 mL polyethylene sample bottle should be included for each sample. Before leaving for the field, each bottle should be filled with 25 mL of reagent grade ethanol, tightly capped, and marked along the fill line. Before use in extraction, the line should be checked to ensure that no ethanol has spilled or evaporated.

For sestonic samples, both chemical and mechanical extraction are used. For chemical extractions, a sufficient quantity of buffered acetone should be kept in supply. After chemical extractant is added, the sample is mechanically extracted either by manual use of a glass mortar and pestle or with an automated grinder. Extracted samples are placed in 13 mL screw cap vials. All extraction equipment should be cleaned thoroughly before and after each use, a laboratory blank (code 32) should be collected when samples are processed.

6.0 Collection of Chlorophyll-a Samples

6.1 Benthic Sampling

Following is a detailed description of sampling procedures. Because sampling sequence is important, please follow the protocol as outlined. The general methodology underpinning periphyton sampling involves collecting and compositing samples taken at equidistant transects along a representative reach. Within this reach, samples will be collected in several representative habitats—erosional and depositional. Erosional habitats include riffles and runs. Depositional habitats are slack water and are mostly contained within pooling areas. In order to collect a representative sample within each stream reach, each type of habitat should be sampled. The sampling sequence will include the following generalized steps:

1. Establishment of reach and transects
2. Collection of samples
3. Extraction of samples

6.11 Establishment of reach and transects

The stream reach is defined as 20 times the wetted width of the widest section. Along this reach, 11 equidistant transects are sampled in an effort to sample all represented habitats. To establish, follow these steps:

a. To establish the sampled stream reach, measure the widest wetted width in meters and multiply by 20 (e.g., widest wetted width = 11 meters; stream reach = 20 x 11 meters = 220 meters).

b. To establish 11 equidistant transects (A-K), divide the total stream reach by 10 (e.g., stream reach = 220 meters; transect width = 220 meters/10 = 22 meters). Using the previous example, transect A will be at the head of the stream reach (0 meters), transects B-J will be at 22 meter intervals, and transect K will be at the bottom of the stream reach (220 meters).

6.12 Collection of Samples

At each transect, the sample will be collected at left (L), right (R), or center (C). The type of substrate—soft or hard, will determine the method used to collect the sample. Soft substrates include sand, silt and gravel. Hard substrates are all materials larger than gravel including hardpan and bedrock. **Please keep composited sample out of sunlight.** To collect, follow these steps:

a. The sampling point for each transect is established randomly at the first transect. Several methods may be used to randomize the first point, but the most available method involves the second hand on a watch. By looking at the second hand, "L" is represented by 1-20, "C" by 21-40, and "R" by 41-60. Each following sampling point is established by going from left bank to right bank, back to the left bank and on to the right bank. Repeat this process until all transects are assigned a sampling point.
b. Sampling moving upstream, determine the most representative habitat type at each transect and sample that habitat. Sampling technique will be dependent upon substrate type. Always pick an accessible collection area along transect. Collection area should be as close to the center of the sampling point as possible. Do not sample depths deeper than mid-bicep. Composite samples from each point into 1or 2-gallon wide mouth jar.
c. Sampling Soft Substrates
   - Place capped delimiter over substrate, pressing in until pressure is felt on cap.
   - Slide spatula underneath delimiter and lift sample from water ensuring that none of the sample is lost.
   - Pour sample into 1-gallon jar using small funnel. Using native water, rinse the delimiter, spatula and funnel.
d. Sampling Removable Hard Substrates (e.g., cobble)
   - Remove rock(s) from stream and place open delimiter over substrate to define sampling area. Outline sampling area with a sharp edge.
   - Place rock(s) into large funnel and scrub delimited area with a medium coarse wire brush. Periodically wash scrubbed area into jar and continue scrubbing until all periphyton has been removed from rock.
e. Sampling Unremoveable Hard Substrates (e.g., hardpan)
   - Place capped delimiter over substrate, pressing in until pressure is felt on cap.
   - Slide spatula underneath delimiter and lift sample from water ensuring that none of the sample is lost.

- Pour sample into 1-gallon jar using small funnel. Using native water, rinse the delimiter, spatula and funnel.

6.13 Extraction of Samples

Extraction method is 48 hours in ethanol at ambient temperature. Mechanical extraction is not used. The following steps are used to extract the subsample.

- A subsample of 25 mL from a completely mixed composite is measured using a graduated cylinder.
- The subsample is filtered using a glass or fiber filter (0.45μm porosity, 47-mm diameter). Filtration should not occur above 20 psi.
- When entire subsample is filtered, the filter is removed from the unit and placed in 25 mL of ethanol. The ethanol should be in a wrapped 100 mL polyethylene bottle.
- Sample is transported in the cab of the truck and should be delivered to the laboratory within 48 hours.

6.2 Sestonic Samples

Sestonic chlorophyll sampling and post-processing for streams is described in the lakes portion of the monitoring SOP. For streams, the only sampling difference will be the collection of a composited, depth-integrated sample from the splitter churn. This will be done while general chemistry samples are being aliquoted. Water collected for chlorophyll-a analysis has a 24 hour holding time and should be processed immediately in the field. Light and heat degrade chlorophyll, so it is imperative to minimize exposure to heat and sunlight and artificial light (i.e. don't process outside in direct sunlight, keep ice chest lids closed tightly). Chlorophyll-a must be filtered immediately after exposure to light to avoid degradation. Chlorophyll-a filtrates must be wrapped in foil, labeled, bagged and frozen on ice immediately upon processing. These filtrates may be kept frozen for up to 30 days before extraction occurs. Extracts must also be frozen immediately after preparation and should be submitted to the lab for analysis within one month of being processed.

6.3 Photo Documentation

7.0 Forms

7.1 Field Notes

Field notes are documents used to annotate and record information that is gathered at the project site. They are a data sheet and should be treated as such. Therefore, they should be written, legible, and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. Field notes should be initialed and dated by the collecting personnel and data entry personnel. For guidance on proper procedure to complete the field notes, refer to your supervisor and or FTE. Field notes can be found at S:\Monitoring\STREAMS\forms\Field Notes.doc.

7.2 Laboratory Log-in Sheets

Log-in sheets are documents turned into the analytical laboratory for each sample collected. These forms are used to denote the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time of sample collection and be legible and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. For guidance on proper procedure to complete the log-in sheets, refer to your supervisor and or FTE. Log-in sheets can be found at S:\Monitoring\STREAMS\forms\.

7.3 Chains of Custody

Chains of custody are documents turned into the analytical laboratory for each group of samples collected. These forms are used for several purposes. They act as a legal document to show proper delivery of samples occurred and they make a general list of the parameters that should be analyzed. Chains of custody are available for inorganic, metals, and organics panels. They are a data sheet and should be treated as such. Therefore, they should include the date and time for each sample collected and be legible and complete. They should also be signed and dated by field and laboratory receiving personnel at the time of delivery. To avoid confusion and loss of data, a new chain of custody should be used for each group of samples. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and or FTE. Chains of custody can be found at S:\Monitoring\STREAMS\forms\.

8.0 Data Storage

All completed paper copies of forms and data sheets should be maintained with the appropriate station notebook. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Water Quality Database. Each sample should be maintained electronically in the database under a unique sample number.

9.0 References

Hill, B. H., "Periphyton", EMAP Western Pilot Study Field Operations Manual for Wadeable Streams, 156-158 (2001).

APPENDIX 2

Photodocumentation of Periphyton Collected Summer 2003

07/29/03 GLOVER RIVER NEAR GLOVER 28 mg/m^2

07/29/03 MOUNTAIN FORK AT SMITHVILLE 8 mg/m^2

AUG 05/ 03 CANADIAN RIVER AT KONOWA 13 mg/m^2

AUG 05/ 03 CANADIAN RIVER AT KONOWA 13 mg/m^2

AUG 05/ 03 CANADIAN RIVER AT KONOWA

AUG 05/ 03 CANADIAN RIVER AT PURCELL 25 mg/m^2

NORTH CANADIAN AT EL RENO AUG 6, 2003 < 1 mg/m^2

NORTH CANADIAN AT EL RENO AUG 6, 2003

HONEY CREEK, OFF SH 25, GROVE 26-AUG-03 44 mg/m^2

HONEY CREEK, OFF SH 25, GROVE 26-AUG-03 44 mg/m^2

HONEY CREEK, OFF SH 25, GROVE 26-AUG-03 44 mg/m^2

ELK RIVER 8/26 40 mg/m^2